Title: Clinical and pathological features of combined hepatocellular-cholangiocarcinoma compared with other liver cancers

Authors: Kazuki Wakizaka, Hideki Yokoo, Toshiya Kamiyama, Masafumi Ohira, Koichi Kato, Yuki Fujii, Ko Sugiyama, Naoki Okada, Takanori Ohata, Akihisa Nagatsu, Shingo Shimada, Tatsuya Orimo, Hirofumi Kamachi, Akinobu Taketomi

Affiliations: Department of Gastroenterological Surgery I, Hokkaido University Graduate School of Medicine, Sapporo, Japan

Corresponding author:

Kazuki Wakizaka, Department of Gastroenterological Surgery I, Hokkaido University Graduate School of Medicine, N15 W7 Kita-ku, Sapporo 060-8638, Japan.

Tel: +81-11-706-5927

Fax: +81-11-717-7515

E-mail: kazuki_ip_go_go@yahoo.co.jp

Disclosure statement: The authors have no conflicts of interest to disclose, including financial disclosures, and no grants from any organizations.

Acknowledgment: We thank Susan Furness, PhD, from Edanz Group (www.edanzediting.com/ac) for editing a draft of this manuscript.

Title:

Clinical and pathological features of combined hepatocellular-cholangiocarcinoma compared with other liver cancers

Abstract:

Background and Aim: Combined hepatocellular-cholangiocarcinoma (CHC) is a primary liver cancer containing both hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC) elements. Its reported clinicopathological features and prognoses have varied because of its low prevalence. This study aimed to clarify these aspects of CHC.

Methods: We enrolled 28 patients with CHC, 1050 with HCC, and 100 with ICC and compared the clinicopathological characteristics and prognosis of CHC with HCC and ICC. We also analyzed prognostic factors, recurrence patterns, and management in CHC patients.

Results: The incidences of hepatitis B virus (HBV), and high alpha-fetoprotein (AFP) and protein induced by vitamin K absence or antagonists-II (PIVKAII) levels were significantly higher among CHC compared with ICC patients. Multiple tumors were more frequent in CHC compared with the other groups, while vascular invasion and lymph node metastasis were more frequent in the CHC than the HCC group. The 5-year overall (OS) and disease-free survival (DFS) rates for CHC were 25.1% and 22.6%, respectively. OS was significantly lower than for HCC (P<0.001) but not ICC (P=0.152), while DFS was significantly lower than for HCC and ICC (P=0.008 and P=0.005, respectively). Multivariate analysis identified carcinoembryonic antigen (CEA) levels and tumor size as independent predictors in patients with CHC.

Conclusions: The clinical features of CHC, including sex, HBV infection, AFP and PIVKAII levels, were similar to HCC, while its prognosis and pathological features, including vascular invasion and lymph node metastasis, were similar to ICC. CEA levels and tumor size were independent prognostic factors in patients with CHC.

Key words: combined hepatocellular-cholangiocarcinoma, hepatocellular carcinoma, intrahepatic cholangiocarcinoma, resection, prognosis

Abbreviations

CHC, combined hepatocellular-cholangiocarcinoma; HCC, hepatocellular carcinoma; ICC, intrahepatic cholangiocarcinoma; AFP, alpha-fetoprotein; PIVKAII, protein induced by vitamin K absence or antagonists-II; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; OS, overall survival; DFS, disease-free survival; HBV, hepatitis B virus; HR, hazard ratio; CI, confidence interval; HPC, hepatic progenitor cell.

Introduction

Combined hepatocellular-cholangiocarcinoma (CHC) is a rare primary liver cancer containing elements of both hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC), and accounting for 1.0%–4.7% of all primary liver cancers.[1-6] The definition of CHC has changed over time. In 1949, Allen and Lisa classified CHC into double cancer (type A), combined type (type B), and mixed type (type C).[7] In 1985, Goodman et al. modified the classification by dividing CHC into collision (type I), transitional (type II), and fibrolamellar tumors (type III).[8] In 2010, the fourth edition of the World Health Organization (WHO) classification defined two types of CHC: classical type and subtypes with stem-cell features,[9] the latter of which was subdivided into typical, intermediate-cell, and cholangiolocellular subtypes. However, the reported clinical and pathological features and prognoses of CHC have varied because of its low prevalence. In this study, we aimed to compare the clinicopathological features and prognosis of CHC with those of HCC and ICC, and to analyze the prognostic factors in patients with CHC.

Patients and Methods

A search of patients who underwent hepatic resection for primary liver cancer at our institution between January 1990 and March 2016 identified 28 patients with CHC, 1050 with HCC, and 100 with ICC. CHC was diagnosed according to the definition of type C in Allen and Lisa's classification; i.e., double cancer and collision cancer were excluded (Fig. 1).[7]

Patients were followed-up postoperatively on an outpatient basis by monitoring of tumor markers and abdominal dynamic computed tomography and/or magnetic resonance imaging every 3 months. Recurrence of CHC, HCC, or ICC was diagnosed by combined examination of imaging studies and tumor markers.

The medical records of all patients were reviewed for clinicopathological information, including sex, age, viral markers, serum alpha-fetoprotein (AFP), protein induced by vitamin K absence or antagonists-II (PIVKAII), carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9), surgical procedure, and pathological reports. Pathological data included tumor number, tumor size, lymph node metastasis, vascular invasion, and the findings of non-cancerous liver. Tumor node metastasis (TNM) stage was determined according to the criteria of the Liver Cancer Study Group of Japan (6th edition).[10] We compared the clinical and pathological characteristics, overall survival (OS), and disease-free survival (DFS) between patients with CHC and patients with HCC or ICC. We also investigated the prognostic factors for OS and DFS, recurrence patterns, and management among patients with CHC. This study was approved by the institutional review board of our institution.

Statistical analysis

Statistical analyses were performed using EZR version 1.35 (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria).[11] Clinical and pathological variables were examined by Fisher's exact test and Student's *t*-test. OS and DFS curves were plotted using the Kaplan–Meier method and compared using the log-rank test. Multivariate analyses were performed using the Cox proportional hazards regression model. A *P* value of <0.05 was considered statistically significant.

Results

Clinical and pathological characteristics

The 28 patients with CHC included 23 males and five females, with a mean age of 62.5±11.8 years (range, 32–78 years). There was no significant difference in sex ratio or age between the CHC group and the HCC/ICC group. Eleven patients (39.3%) were positive for hepatitis B virus (HBV) and five (17.9%) were positive for hepatitis C virus (HCV). Significantly more patients in the CHC group had HBV infection compared with the ICC group. All patients in the CHC group were classified as Child-Pugh A. Above normal levels of preoperative AFP (≥10 ng/ml), PIVKAII (≥40 mAU/ml), CEA (≥6.5 ng/ml), and CA19-9 (≥37 U/ml) were found in 18 (64.3%), 14 (50.0%), seven (25.0%), and 14 (50.0%) CHC patients, respectively. Significantly more patients in the CHC group had elevated AFP and PIVKAII levels compared with the ICC group. Regarding the surgical procedures, anatomical resection was performed in 28 (87.5%) CHC patients, 691 (65.8%) HCC patients, and 95 (95.0%) ICC patients. Anatomical resection was performed significantly more frequently in patients with CHC than HCC. The resection surface was histologically free of tumor in all patients. Multiple tumors were seen in 16 patients (57.1%) with CHC, which was significantly more than in the other groups. Tumor size was not significantly different among the groups. Vascular invasion, especially portal vein invasion, was more frequent in the CHC compared with the HCC group but not the ICC group; however, hepatic vein invasion or bile duct invasion was significantly less frequent in the CHC compared with the ICC group. All patients with CHC underwent lymph node dissection, and lymph node metastasis was more frequent in the CHC compared with the HCC group. TNM stage was worse in the CHC compared with the HCC group, but there was no significant difference between the CHC and ICC

groups. Pathologically, patients with CHC had significantly less cirrhosis than patients with HCC, but more than patients with ICC (Table 1).

Survival analysis

The survival analysis data are summarized in Table 2. The median OS in the CHC group was 25 months, and the 1-, 3-, and 5-year OS rates were 57.9%, 33.4%, and 25.1%, respectively. OS in the CHC group was significantly lower than in the HCC group (P<0.001) but not significantly different from the ICC group (P=0.152) (Fig. 2A). The median DFS was 5 months, and the 1-, 3-, and 5- DFS rates were 30.2%, 22.6%, and 22.6%, respectively. DFS was significantly lower in the CHC group compared with both the HCC and ICC groups (P=0.008 and P=0.005, respectively) (Fig. 2B). We compared the prognoses of CHC and HCC according to stage, and showed that OS tended to be lower in the CHC compared with the HCC group among stage III and IVa patients, but there was no significant difference for any stage. Similarly, there was no significant difference in DFS between the CHC and HCC groups at any stage (Fig. S1).

Prognostic factor analysis

Univariate analysis showed that CEA level, tumor size, and vascular invasion were predictive factors for lower OS in patients with CHC, while CEA level and tumor size were also predictive factors for lower DFS in CHC patients. Multivariate analysis showed that CEA level and tumor size were independent predictors of OS (CEA: hazard ratio (HR) 3.35, 95% confidence interval (CI) 1.06–10.56, P=0.039; tumor size: HR 2.69, 95% CI 1.04–6.92, P=0.041) and recurrence (CEA: HR 3.17, 95% CI 1.13–8.88, P=0.028; tumor size: HR 3.14, 95% CI 1.30–7.59, P=0.011) in patients with CHC (Table 3).

Recurrence of CHC and management

The recurrence pattern of CHC and its management are summarized in Table 4. At the date of our investigations, 22 of the 28 patients with CHC (78.6%) had experienced recurrences, with a median time to recurrence of 5 months (range 0.2–67). Twelve of the 22 patients had extrahepatic recurrences, including lymph node metastasis in seven patients, lung metastasis in six, bone metastasis in one, and brain metastasis in one. The management strategies for the first recurrence varied, including resection for intrahepatic recurrence or solitary lymph node metastasis, resection, radiofrequency ablation, transcatheter arterial chemoembolization, or chemotherapy for intrahepatic recurrence, and radiotherapy for bone metastasis. Extrahepatic recurrence was mainly treated with chemotherapy, including fluorouracil and cisplatin (n=4), tegafur/uracil (n=2), tegafur/gimeracil/oteracil (n=2), fluorouracil (n=1), gemcitabine (n=1), tegafur/gimeracil/oteracil and gemcitabine (n=1), and sorafenib (n=1).

The clinicopathological features in patients with intrahepatic and extrahepatic recurrence are summarized in Table 5. Recurrence only in the liver occurred in 10 patients and liver recurrence together with extrahepatic recurrence occurred in 12 patients. Extrahepatic recurrence was significantly correlated with tumor size, lymph node metastasis, and stage (P=0.002, P=0.015, and P=0.012, respectively). OS rates after intrahepatic and extrahepatic recurrence are shown in Fig. S2. The 1-year OS rate in patients with extrahepatic recurrence tended to be poorer than in those with intrahepatic recurrence, but there was no significant difference (P=0.562).

Discussion

CHC is a rare type of primary liver cancer, accounting for 1.0%–4.7% of primary liver cancers.[1-6] In our institution, 28 of 1,178 patients (2.4%) who underwent resection for primary liver cancer had CHC, which was similar to the incidence in previous reports.[1-6] However, the definition of CHC has changed, with both the Allen and Lisa classification and Goodman classification being used in clinical practice. The current definition of CHC is Allen and Lisa type C or Goodman type II.[7,8] Although the latest WHO classification has made the definition of CHC clearer, it has not been easily accepted by clinicians because of its complexities regarding subtypes with stem-cell features.[9]

Although the origin of CHC cells remains unclear, CHC is widely considered to be derived from hepatic progenitor cells (HPCs).[5,12-15] HPCs have bipotential differentiation into either hepatocytes or cholangiocytes, which are thought to be located near the canal of Hering.[12,16] HPCs are induced and expanded during liver injury, such as hepatitis or cirrhosis, leading to malignant transformation, which is thought to be a mechanism for carcinogenesis of CHC. [17]

The clinical and pathological features of CHC are similar to those of HCC or ICC. The current study found no difference in age at diagnosis among the three groups. Male patients were predominant in all groups, and although the male-to-female ratios in CHC and HCC differed from that in ICC, the difference was not significant. HBV infection was significantly more common among patients with CHC or HCC compared with ICC. These results were largely in accord with previous reports indicating similar tumorigenic backgrounds for CHC and HCC.[18,19] The incidences of elevated AFP, PIVKAII, CEA, and CA19-9 in patients with CHC were 64.3%, 50.0%, 25.0%, 50.0%,

respectively, which were similar to those reported in previous studies.[18-21] The rates of elevated AFP and PIVKAII were similar in CHC and HCC patients, but were significantly lower in patients with ICC, while the rates of elevated CEA were similar in CHC and ICC patients and lower in HCC patients. The levels of these tumor markers support the idea that CHC includes both HCC and ICC components. The frequency of lymph node metastasis in CHC was 21.4%, which was in accord with incidences reported in previous studies (12%–33%).[19,22,23]

CHC is difficult to diagnose preoperatively due to its heterogeneous imaging characteristics, with features that overlap both HCC and ICC.[24] Thus most CHC cases are initially misdiagnosed as either HCC or ICC, and the correct diagnosis is only reached after examination of surgical specimens. Some authors have suggested that CHC should be considered preoperatively in patients with tumors showing characteristic imaging features of HCC but elevated CA19-9 levels, tumors with characteristic ICC imaging features and elevated AFP levels, or in patients with elevated levels of both serum markers. [25,26]

Minor or major hepatic resection, with or without lymph node dissection, is currently the consensus recommended treatment for CHC.[24,27] The outcomes following liver transplantation are consistently poorer in patients with CHC compared with HCC,[28,29] and one study found no survival benefit of transplant over resection in patients with CHC.[30] Although some studies have reported a good response of CHC to transarterial chemoembolization and sorafenib, these treatments require further evaluation.[27,31,32]

Survival of patients with CHC has been reported in several previous studies, with a 5-year OS rate of 7.9%–36.4%.[1,21-23] Previous studies consistently found that

CHC had a poorer prognosis than HCC,[1,2,21-23,33] while the prognosis with regard to ICC varied, with some reports showing a poorer or similar prognosis and others showing a better prognosis.[1,2,21-23,33] Our results showed that the OS of patients with CHC was significantly poorer than that of patients with HCC but not significantly different from patients with ICC, while CHC was associated with a significantly poorer DFS than either HCC or ICC. Adverse clinicopathological prognostic factors associated with tumor recurrence and survival have been reported in several studies, and included large tumor size (>5 cm), presence of satellite nodules, lymph node involvement, multifocality, vascular invasion, portal vein invasion, high tumor stage, high levels of CA19-9, decreased capsule formation, and free surgical margins <2 cm.[18,24,34,35] However, many of these factors were not significant according to multivariate analysis. Our results identified tumor size ≥5 cm and high levels of CEA as significant prognostic factors for OS and DFS according to multivariate analysis.

Few studies have reported on the recurrence of CHC after resection. One study found that CHC recurred frequently in the liver, while another found that extrahepatic recurrence was commonly seen in lymph nodes.[1,19,21] The present study showed that extrahepatic metastasis was the most frequent form of recurrence, and was managed by chemotherapy. Furthermore, extrahepatic recurrence was correlated with tumor size, lymph node metastasis, and stage. There are currently no definitive guidelines for the treatment of recurrent CHC, and chemotherapy regimens were chosen according to the treatment of HCC or ICC; however, the outcomes after recurrence were poor. The prognosis of CHC after resection is thus poor due to both the high recurrence rate, including extrahepatic recurrence, and ineffective treatment after recurrence. In practice, adjuvant postoperative chemotherapy might be a useful strategy in patients

with CHC, especially in patients at high risk of extrahepatic recurrence due to tumor size ≥5 cm, lymph node metastasis, and poor stage.

This study had some limitations. First, it was a retrospective, single-center study, and second, the sample size for CHC was relatively small. Further, high-volume, multicenter studies are therefore necessary to validate our results.

In conclusion, the clinical features of patients with CHC, including sex, HBV infection, AFP levels, and PIVKAII levels, were more similar to HCC than ICC, while its prognosis and pathological features, including vascular invasion and lymph node metastasis, were more similar to ICC. Multivariate analysis identified high CEA levels and tumor size ≥5 cm as independent prognostic factors for both OS and recurrence in patients with CHC.

References

1 Jarnagin WR, Weber S, Tickoo SK, Koea JB, Obiekwe S, Fong Y, et al. Combined hepatocellular and cholangiocarcinoma: demographic, clinical, and prognostic factors. Cancer 2002; 94: 2040-2046.

2 Liu CL, Fan ST, Lo CM, Ng IO, Lam CM, Poon RT, et al. Hepatic resection for combined hepatocellular and cholangiocarcinoma. Arch Surg 2003; 238: 86-90.

3 Lee WS, Lee KW, Heo JS, Kim SJ, Choi SH, Kim YI, et al. Comparison of combined hepatocellular and cholangiocarcinoma with hepatocellular carcinoma and intrahepatic cholangiocarcinoma. Surg Today 2006; 36: 892-897.

4 Portolani N, Baiocchi GL, Coniglio A, Piardi T, Grazioli L, Benetti A, et al. Intrahepatic cholangiocarcinoma and combined hepatocellular-cholangiocarcinoma: a Western experience. Ann Surg Oncol 2008; 15: 1880–1890.

5 Yeh MM. Pathology of combined hepatocellular-cholangiocarcinoma. J Gastroenterol Hepatol 2010; 25: 1485–1492.

6 Yoon YI, Hwang S, Lee YJ, Kim KH, Ahn CS, Moon DB, et al. Postresection Outcomes of Combined Hepatocellular Carcinoma-Cholangiocarcinoma, Hepatocellular Carcinoma and Intrahepatic Cholangiocarcinoma. J Gastrointest Surg 2016; 20: 411–420.

7 Allen RA, Lisa JR. Combined liver cell and bile duct carcinoma. Am J Pathol 1949; 25: 647–655.

8 Goodman ZD, Ishak KG, Langloss JM, Sesterhenn IA, Rabin L. Combined hepatocellular-cholangiocarcinoma. A histologic and immunohistochemical study. Cancer 1985; 55: 124–135.

9 Theise ND, Nakashima O, Park YN, Nakanuma Y. Combined hepatocellular-cholangiocarcinoma. In: Bosman FT, Carneiro, F, Hruban RH, Theise ND, eds. WHO Classification of Tumours of the Digestive System. Volume 3. 4th ed. Lyon, France: IARC Press, 2010: 225–227.

10 The Liver Cancer Study Group of Japan. The General Rules for the Clinical and Pathological Study of Primary Liver Cancer, 6th edn. Tokyo: KANEHARA & Co., LTD., 2015; p26.

11 Kanda Y. Investigation of the freely-available easy-to-use software "EZR" (Easy R) for medical statistics. Bone Marrow Transplant 2013; 48: 452-458.

12 Theise ND, Yao JL, Harada K, Hytiroglou P, Portmann B, Thung SN, et al. Hepatic 'stem cell' malignancies in adults: four cases. Histopathology 2003; 43: 263–271.

13 Kim H, Park C, Han KH, Choi J, Kim YB, Kim JK, et al. Primary liver carcinoma of intermediate (hepatocyte-cholangiocyte) phenotype. J Hepatol 2004; 40: 298–304.

14 Itoyama M, Hata M, Yamanegi K, Yamada N, Ohyama H, Hirano H, et al. Expression of both hepatocellular carcinoma and cholangiocarcinoma phenotypes in hepatocellular carcinoma and cholangiocarcinoma components in combined hepatocellular and cholangiocarcinoma. Med Mol Morphol 2012; 45: 7–13.

15 Ogasawara S, Akiba J, Nakayama M, Nakashima O, Torimura T, Yano H. Epithelial cell adhesion molecule-positive human hepatic neoplastic cells: development of combined hepatocellular-cholangiocarcinoma in mice. J Gastroenterol Hepatol 2015; 30: 413–420.

16 Theise ND, Saxena R, Portmann BC, Thung SN, Yee H, Chiriboga L, et al. The canals of Hering and hepatic stem cells in humans. Hepatology 1999; 30: 1425–1433.

17 Miyagima A, Tanaka M, Itoh T. Stem/Progenitor Cells in Liver Development, Homeostasis, Regeneration, and Reprogramming. Cell Stem Cell 2014; 14: 561-574.

18 Kim KH, Lee SG, Park EH, Hwang S, Ahn CS, Moon DB, et al. Surgical treatments and prognoses of patients with combined hepatocellular carcinoma and cholangiocarcinoma. Ann Surg Oncol 2009; 16: 623–629.

19 Lee SD, Park SJ, Han SS, Kim SH, Kim YK, Lee SA, et al. Clinicopathological features and prognosis of combined hepatocellular carcinoma and cholangiocarcinoma after surgery. Hepatobiliary Pancreat Dis Int 2014; 13: 594-601.

20 Tang D, Nagano H, Nakamura M, Wada H, Marubashi S, Miyamoto A, et al. Clinical and pathological features of Allen's type C classification of resected combined hepatocellular and cholangiocarcinoma: a comparative study with hepatocellular carcinoma and cholangiocellular carcionoma. J Gastrointest Surg 2006; 10: 987-998.

21 Yano Y, Yamamoto J, Kosuge T, Sakamoto Y, Yamasaki S, Shimada K, et al. Combined hepatocellular and cholangiocarcinoma: a clinicopathologic study of 26 resected cases. Jpn J Clin Oncol 2003; 33: 283–287.

22 Zuo HQ, Yan LN, Zeng Y, Yang JY, Luo HZ, Liu JW, et al. Clinicopathological characteristics of 15 patients with combined hepatocellular carcinoma and cholangiocarcinoma. Hepatobiliary Pancreat Dis Int 2007; 6: 161-165.

23 Yin X, Zhang BH, Qiu SJ, Ren ZG, Zhou J, Chen XH, et al. Combined hepatocellular carcinoma and cholangiocarcinoma: clinical features, treatment modalities, and prognosis. Ann Surg Oncol 2012; 19: 2869-2876.

24 Gera S, Ettel M, Acosta-Gonzalez G, Xu R. Clinical features, histology, and histogenesis of combined hepatocellular-cholangiocarcinoma. World J Hepatol 2017; 9: 300-309.

25 Kassahun WT, Hauss J. Management of combined hepatocellular and cholangiocarcinoma. Int J Clin Pract 2008; 62: 1271-1278.

26 Sanada Y, Shiozaki S, Aoki H, Takakura N, Yoshida K, Yamaguchi Y. A clinical study of 11 cases of combined hepatocellular-cholangiocarcinoma. Assessment of enhancement patterns on dynamics computed tomography before resection. Hepatol Res 2005; 32: 185-195

27 Chi M, Mikhitarian K, Shi C, Goff LW. Management of combined hepatocellular-cholangiocarcinoma: a case report and literature review. Gastrointest Cancer 2012; 5: 199-202

28 Sapisochin G, Fidelman N, Roberts JP, Yao FY. Mixed hepatocellular cholangiocarcinoma and intrahepatic cholangiocarcinoma in patients undergoing transplantation for hepatocellular carcinoma. Liver Transpl 2011; 17: 934–942.

29 Park YH, Hwang S, Ahn CS, Kim KH, Moon DB, Ha TY, et al. Long-term outcome of liver transplantation for combined hepatocellular carcinoma and cholangiocarcinoma. Transplant Proc 2013; 45: 3038–3040.

30 Groeschl RT, Turaga KK, Gamblin TC. Transplantation versus resection for patients with combined hepatocellular carcinoma-cholangiocarcinoma. J Surg

Oncol 2013; 107: 608–612.

31 Kim GM, Jeung HC, Kim D, Kim JH, Yoon SH, Jung ES, et al. A case of combined hepatocellular-cholangiocarcinoma with favourable response to systemic chemotherapy. Cancer Res Treat 2010; 42: 235-238.

32 O'Connor K, Walsh JC, Schaeffer DF. Combined hepatocellular-cholangiocarcinoma (cHCC-CC): a distinct entity. Ann Hepatol 2014; 13: 317–322.

33 Koh KC, Lee H, Choi MS, Lee JH, Paik SW, Yoo BC, et al. Clinicopathologic features and prognosis of combined hepatocellular cholangiocarcinoma. Am J Surg 2005; 189: 120-125.

34 Chu KJ, Lu CD, Dong H, Fu XH, Zhang HW, Yao XP. Hepatitis B virus-related combined hepatocellular-cholangiocarcinoma: clinicopathological and prognostic analysis of 390 cases. Eur J Gastroenterol Hepatol 2014; 26: 192–199.

35 Song S, Moon HH, Lee S, Kim TS, Shin M, Kim JM, et al. Comparison between resection and transplantation in combined hepatocellular and cholangiocarcinoma. Transplant Proc 2013; 45: 3041–3046.

Supporting information

Additional supporting information may be found online in the supporting information section of this article.

Figure S1. Overall survival (OS) (A) and disease-free survival (DFS) rates (B) in patients with combined hepatocellular-cholangiocarcinoma (CHC) and hepatocellular carcinoma (HCC) according to Liver Cancer Study Group of Japan (6th edition) stage. OS tended to be lower in patients with stage III and IVa CHC compared with HCC, but there was no significant difference at any stage. There was no significant difference in DFS between the CHC and HCC groups at any stage.

Figure S2. Overall survival (OS) after recurrence in patients with intrahepatic and extrahepatic recurrence of combined hepatocellular-cholangiocarcinoma. The 1-year OS rate in patients with extrahepatic recurrence tended to be poorer than in those with intrahepatic recurrence, but the difference was not significant (P=0.562).

Table 1 Clinical and pathological characteristics of patients with CHC, HCC, and ICC (n, %)

Characteristics	CHC (n=28)	HCC (n=1050)	ICC (n=100)	*P* value	
				CHC vs HCC	CHC vs ICC
Sex					
Male	23 (82.1)	864 (82.3)	62 (62.0)	1.000	0.069
Female	5 (17.5)	186 (17.7)	38 (38.0)		
Age (yr)	62.5±11.8 (32-78)	62.0±10.6 (18-90)	63.5±11.0 (36-87)	0.813	0.660
Viral infection					
HBV	11 (39.3)	407 (38.8)	15 (15.0)	1.000	0.008*
HCV	5 (17.9)	369 (35.1)	12 (12.0)	0.070	0.528
Child-Pugh classification					
A	28 (100.0)	1015 (96.7)	99 (99.0)	1.000	1.000
B	0 (0.0)	35 (3.3)	1 (1.0)		
AFP (ng/ml)					
<10	10 (35.7)	413 (39.6)	57 (80.3)	0.845	<0.001*
≥10	18 (64.3)	631 (60.4)	14 (19.7)		
PIVKAII (mAU/ml)					
<40	14 (50.0)	294 (35.5)	61 (95.3)	0.159	<0.001*
≥40	14 (50.0)	535 (64.5)	3 (4.7)		
CEA (ng/ml)					
<6.5	21 (75.0)	672 (87.8)	54 (73.0)	0.073	1.000
≥6.5	7 (25.0)	93 (12.2)	20 (27.0)		
CA199 (U/ml)					
<37	14 (50.0)	468 (62.9)	27 (37.0)	0.170	0.263
≥37	14 (50.0)	276 (37.1)	46 (63.0)		
Surgical procedure					
Anatomical resection	28 (87.5)	691 (65.8)	95 (95.0)	0.012*	0.219
Non-anatomical resection	4 (12.5)	359 (34.2)	5 (5.0)		
Tumor number					
Solitary	12 (42.9)	668 (63.6)	76 (76.0)	0.030*	0.002*
Multiple	16 (57.1)	382 (36.4)	24 (24.0)		
Tumor size (cm)	6.2±4.2	5.7±4.5	6.1±3.5	0.608	0.923
<2	4 (14.3)	104 (9.9)	6 (6.0)		
2-5	10 (35.7)	503 (47.9)	34 (34.0)		
5-10	9 (32.1)	284 (27.0)	46 (46.0)		

≥10	5 (17.9)	159 (15.1)	14 (14.0)		
Vascular invasion†	19 (67.9)	336 (32.0)	81 (81.0)	<0.001*	0.194
Portal vein invasion	18 (64.3)	295 (28.1)	50 (50.0)	<0.001*	0.204
Hepatic vein invasion	2 (7.1)	115 (11.0)	34 (34.0)	0.760	0.004*
Hepatic artery invasion	0 (0.0)	4 (0.4)	5 (5.0)	1.000	0.585
Bile duct invasion	2 (7.1)	43 (4.1)	57 (57.0)	0.328	<0.001*
Lymph node metastasis	6 (21.4)	15 (1.4)	40 (40.0)	<0.001*	0.079
pStage†					
I	2 (7.1)	93 (8.9)	2 (2.0)	<0.001*	0.157
II	2 (7.1)	433 (41.2)	22 (22.0)		
III	11(39.3)	316 (30.1)	26 (26.0)		
IVa	13 (46.5)	178 (17.0)	43 (43.0)		
IVb	0 (0.0)	30 (2.9)	7 (7.0)		
Non-cancerous liver					
Cirrhosis	5 (17.9)	321 (33.1)	2 (2.5)	0.008*	0.013*
Non-cirrhosis	23 (82.1)	648 (66.9)	77 (97.5)		

*P value <0.05. †Liver Cancer Study Group of Japan, 6th edition.

CHC, combined hepatocellular-cholangiocarcinoma; HCC, hepatocellular carcinoma; ICC, intrahepatic cholangiocarcinoma; HBV, hepatitis B virus; HCV hepatitis C virus; AFP, alpha-fetoprotein; PIVKAII, protein induced by vitamin K absence or antagonists-II; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9.

Table 2 Median survival time and cumulative survival rates in patients with CHC, HCC, and ICC

Groups	Median OS (months)	1-year OS rate	3-year OS rate	5-year OS rate	Median DFS (months)	1-year DFS rate	3-year DFS rate	5-year DFS rate
CHC (n=28)	25	57.9%	33.4%	25.1%	5	30.2%	22.6%	22.6%
HCC (n=1050)	82	87.6%	70.8%	57.8%	23	64.0%	39.7%	30.1%
ICC (n= 100)	37	75.9%	50.9%	41.9%	18	57.7%	41.8%	36.9%

CHC, combined hepatocellular-cholangiocarcinoma; HCC, hepatocellular carcinoma; ICC, intrahepatic cholangiocarcinoma; OS, overall survival; DFS, disease-free survival.

Table 3 Univariate and multivariate analyses of prognostic factors in patients with CHC

Variables	Overall survival			Disease-free survival		
	Univariate analysis	Multivariate analysis		Univariate analysis	Multivariate analysis	
	P value	HR (95%CI)	*P* value	*P* value	HR (95%CI)	*P* value
Gender	0.205			0.132		
Age ≥60 yr	0.083			0.150		
Viral infection	0.346			0.203		
AFP ≥10 ng/ml	0.311			0.605		
PIVKAII ≥40 mAU/ml	0.315			0.240		
CEA ≥6.5 ng/ml	0.008*	3.35 (1.06-10.56)	0.039*	0.009†	3.17 (1.13-8.88)	0.028*
CA19-9 ≥37 U/ml	0.319			0.221		
Surgical procedure	0.644			0.886		
Tumor number	0.633			0.915		
Tumor size ≥5cm	0.006*	2.69 (1.04-6.92)	0.041*	0.004†	3.14 (1.30-7.59)	0.011*
Lymph node metastasis	0.104			0.310		
Vascular invasion	0.023*	2.11 (0.73-6.08)	0.168	0.066		
Pathological cirrhosis	0.219			0.358		

*P value <0.05.

CHC, combined hepatocellular-cholangiocarcinoma; HR, hazard ratio; CI, confidence interval; AFP, alpha-fetoprotein; PIVKAII, protein induced by vitamin K absence or antagonists-II; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9.

Table 4. Recurrence patterns and management of first recurrence after resection of CHC

Variables	n (%)
Number of recurrence	22 (78.6)
Location	
Intrahepatic	10 (45.5)
Extrahepatic	6 (27.3)
Both	6 (27.3)
Management	
Chemotherapy	12 (54.6)
Resection	3 (13.6)
TACE	3 (13.6)
BSC	2 (9.1)
RFA	1 (4.6)
Radiotherapy	1 (4.6)

CHC, combined hepatocellular-cholangiocarcinoma; TACE, transcatheter arterial chemoembolization; BSC, best supportive care; RFA, radiofrequency ablation.

Table 5. Recurrence patterns and characteristics of patients with CHC (n, %)

Characteristics	Recurrence patterns		*P* value
	Intrahepatic (n=10)	Extrahepatic (n=12)	
Sex			
Male	8 (80.0)	11 (91.7)	0.571
Female	2 (20.0)	1 (8.3)	
Age (yr)	62.3±(13.9)	60.3±(10.0)	0.692
Viral infection			
HBV	7 (70.0)	6 (50.0)	0.415
HCV	1 (10.0)	2 (16.7)	1.000
AFP (ng/ml)			
<10	3 (30.0)	5 (41.7)	0.675
≥10	7 (70.0)	7 (58.3)	
PIVKAII (mAU/ml)			
<40	6 (60.0)	4 (33.3)	0.391
≥40	4 (40.0)	8 (66.7)	
CEA (ng/ml)			
<6.5	7 (70.0)	8 (66.7)	1.000
≥6.5	3 (30.0)	4 (33.3)	
CA199 (U/ml)			
<37	2 (20.0)	7 (58.3)	0.099
≥37	8 (80.0)	5 (41.7)	
Surgical procedure			
Anatomical resection	9 (90.0)	10 (83.3)	1.000
Non-anatomical resection	1 (10.0)	2 (16.7)	
Tumor number			
Solitary	5 (50.0)	4 (33.3)	0.666
Multiple	5 (50.0)	8 (66.7)	
Tumor size (cm)			
<5	8 (80.0)	1 (8.3)	0.002*
≥5	2 (20.0)	11 (91.7)	
Vascular invasion†			
No	4 (40.0)	2 (16.7)	0.348
Yes	6 (60.0)	10 (83.3)	
Lymph node metastasis			
No	10 (100.0)	6 (50.0)	0.015*

Yes	0 (0.0)	6 (50.0)	
pStage†			
I	1 (10.0)	0 (0.0)	0.012*
II	1 (10.0)	0 (0.0)	
III	6 (60.0)	2 (16.7)	
Iva	2 (20.0)	10 (83.3)	
Non-cancerous liver			
Cirrhosis	1 (10.0)	2 (16.7)	1.000
Non-cirrhosis	9 (90.0)	10 (83.3)	

*P value <0.05. †Liver Cancer Study Group of Japan, 6th edition.

CHC, combined hepatocellular-cholangiocarcinoma; HBV, hepatitis B virus; HCV hepatitis C virus; AFP, alpha-fetoprotein; PIVKAII, protein induced by vitamin K absence or antagonists-II; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9.

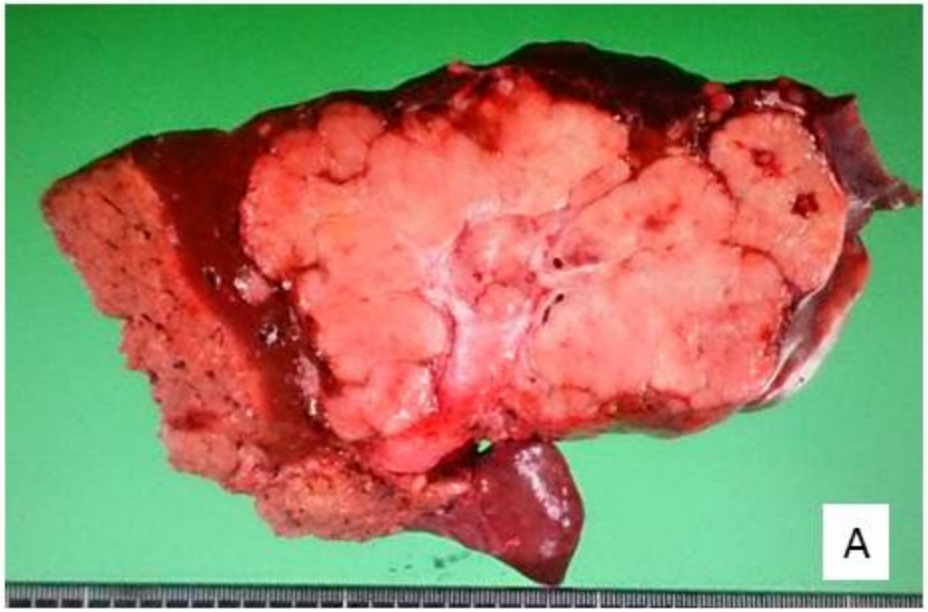
Figure legends

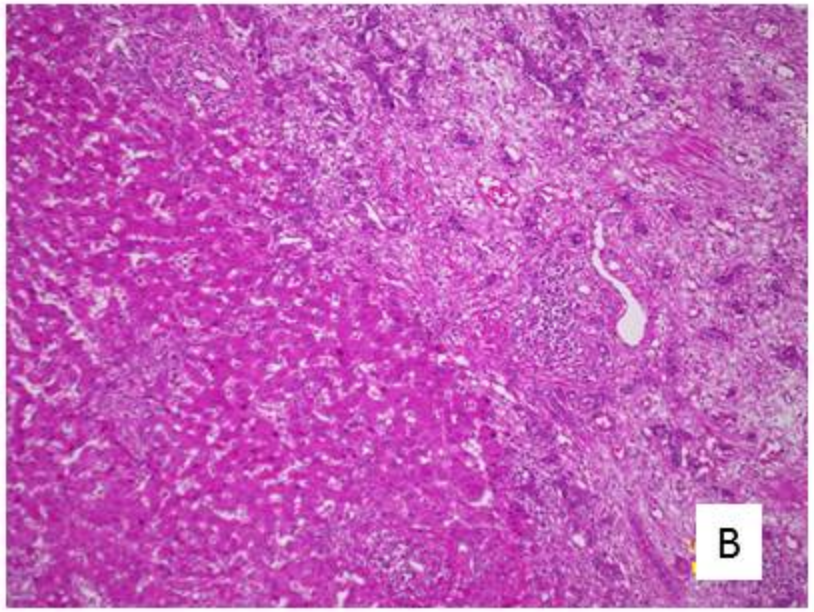
Figure 1

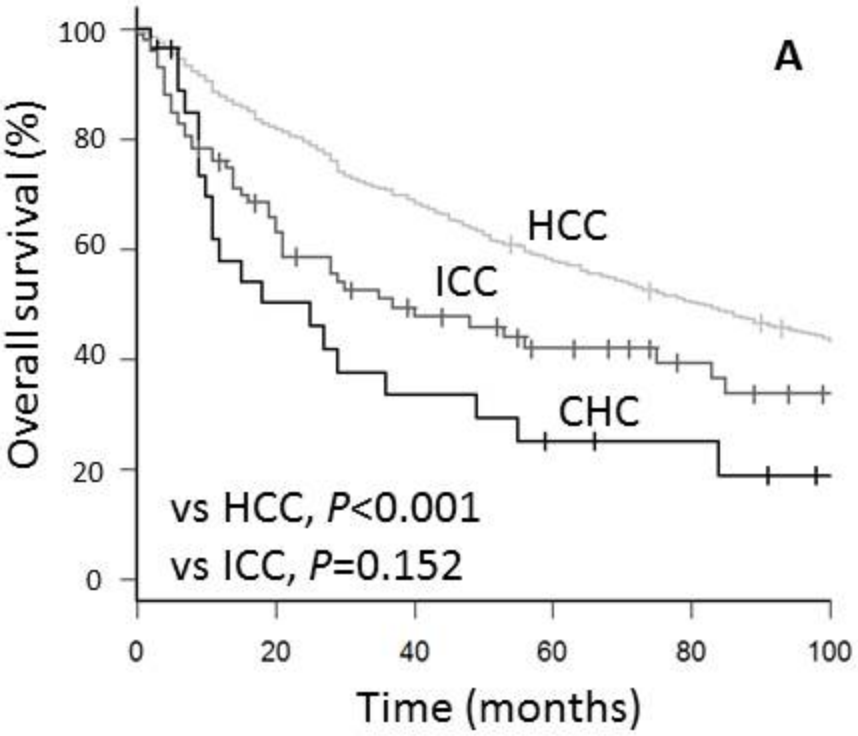
Gross specimen (A) and light microscopy (B; magnification ×40) of combined hepatocellular-cholangiocarcinoma. Homogenous tumor was shown by macroscopic examination, but hepatocellular carcinoma (B, left) and intrahepatic cholangiocarcinoma components (B, right) were shown to coexisted by microscopy.

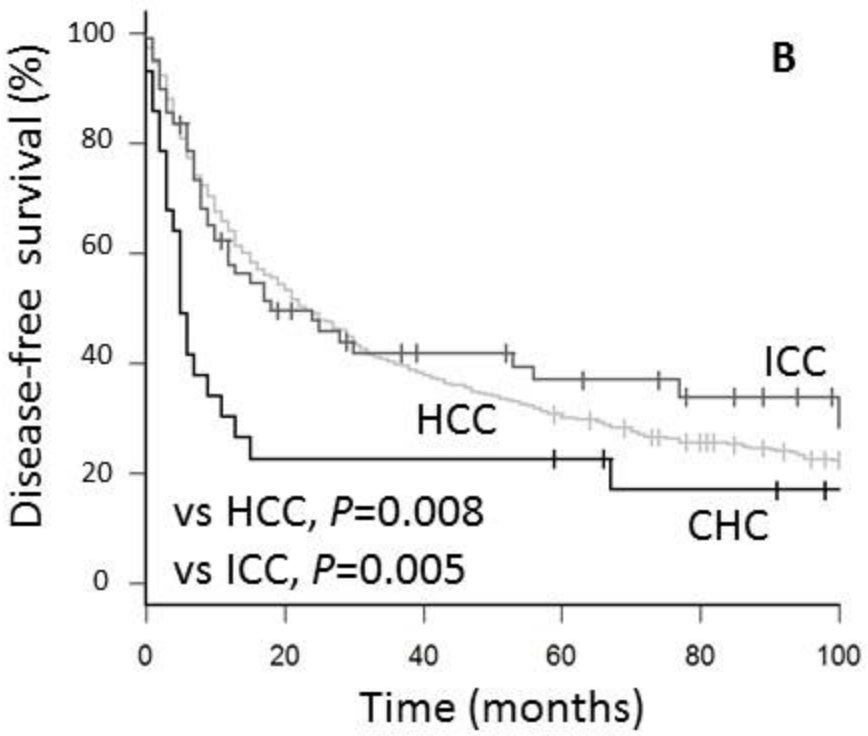
Figure 2

Overall survival (A) and disease-free survival rates (B) of patients with combined hepatocellular-cholangiocarcinoma (CHC), hepatocellular carcinoma (HCC), and intrahepatic cholangiocarcinoma (ICC). Overall survival in the CHC group was significantly lower compared with the HCC group (P<0.001) but not compared with the ICC group (P=0.152). Disease-free survival was significantly lower in the CHC compared with both the HCC and ICC groups (P=0.008, P=0.005, respectively).

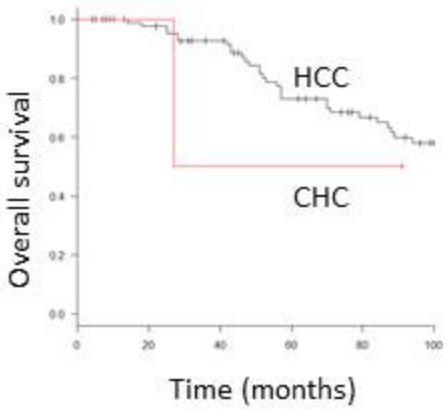

A


B

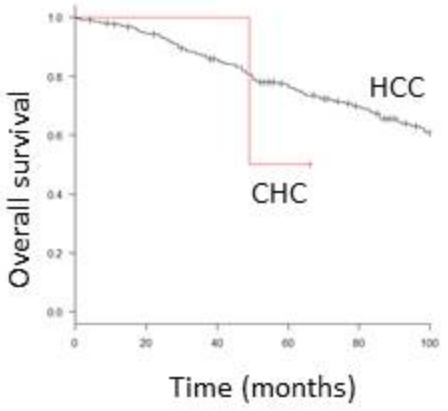

A
Overall survival (%)
100
80
60
40
20
0
HCC
ICC
CHC
vs HCC, P<0.001
vs ICC, P=0.152
0
20
40
60
80
100
Time (months)


B
Disease-free survival (%)
100
80
60
40
20
0
ICC
HCC
CHC
vs HCC, P=0.008
vs ICC, P=0.005
0
20
40
60
80
100
Time (months)

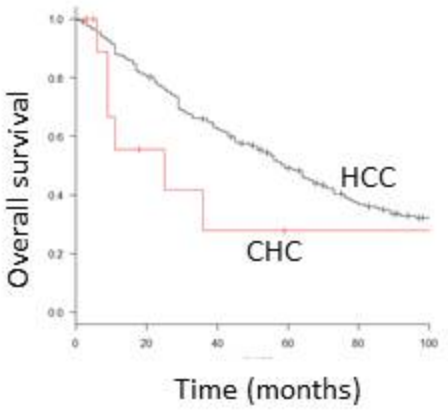
A



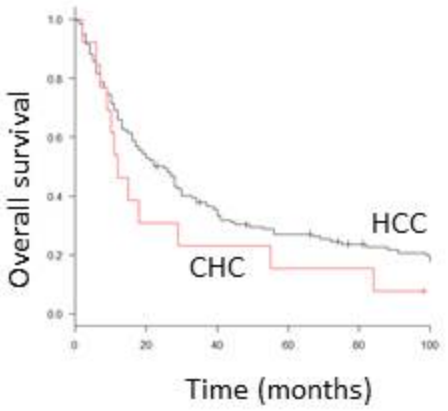
	n	5-year OS rate	*P* value
CHC	2	50.0%	0.562
HCC	93	72.9%	



	n	5-year OS rate	*P* value
CHC	2	50.0%	0.516
HCC	433	76.2%	

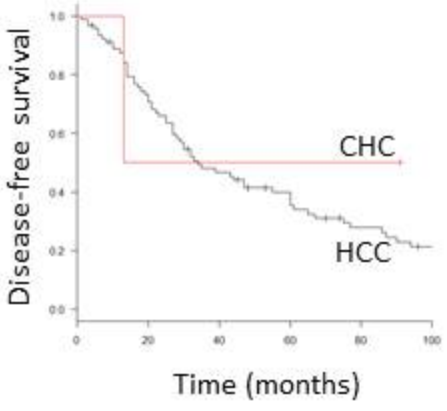


	n	5-year OS rate	*P* value
CHC	11	27.8%	0.121
HCC	316	49.2%	

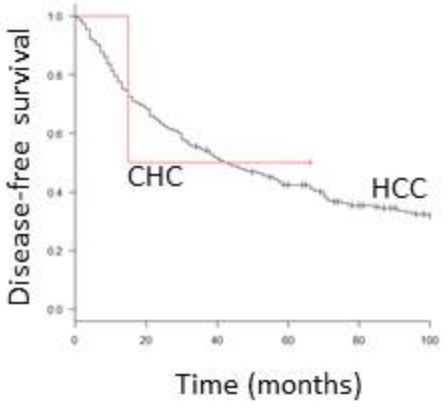


	n	5-year OS rate	*P* value
CHC	13	15.4%	0.180
HCC	178	27.1%	

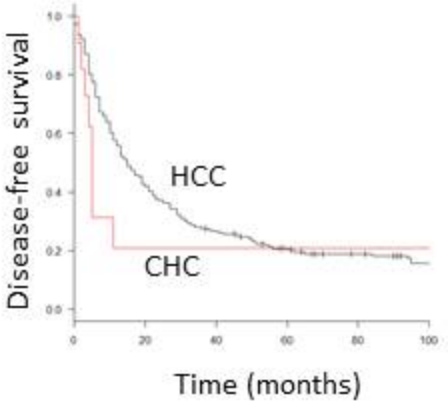
B



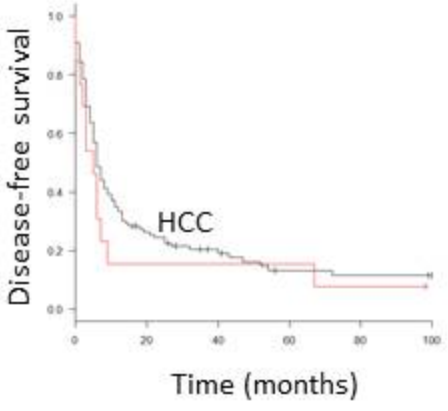
	n	5-year DFS rate	*P* value
CHC	2	50.0%	0.633
HCC	93	35.5%	



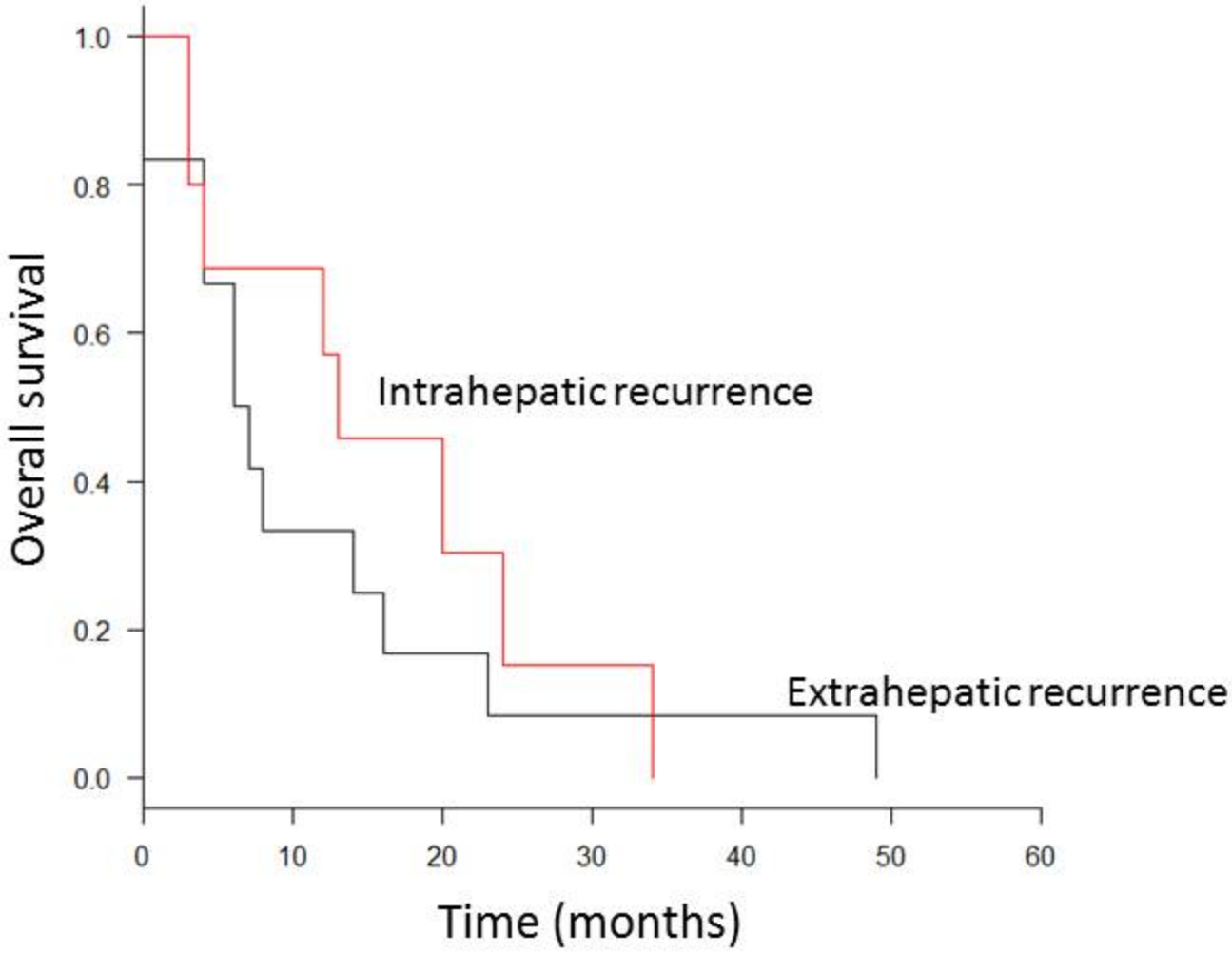
	n	5-year DFS rate	*P* value
CHC	2	50.0%	0.857
HCC	433	42.4%	



	n	5-year DFS rate	*P* value
CHC	11	20.8%	0.236
HCC	316	20.1%	



	n	5-year DFS rate	*P* value
CHC	13	15.4%	0.301
HCC	178	13.2%	



	n	1-year OS rate	*P* value
Intrahepatic recurrence	10	57.1%	0.562
Extrahepatic recurrence	12	33.3%	